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EDITORIAL

THE NEW MILD TINCTURE OF IODINE

The New Pharmacopœia, after a great deal of struggling with nomenclature, has finally adopted a new mild tincture of iodine, which proves, despite the colorful competition which iodine the germicide has had in recent years, that it is still accepted as the outstanding antiseptic of its kind.

Elemental iodine has a distinct property which it does not always confer upon its compounds, except those which freely dissociate. It is the property of attacking germs. Of course, like most germicides, iodine does not always know how to differentiate between a good germ and a bad germ.

One of the facts so often overlooked in considering germicides is that when they are inimical to germs they are also unfriendly to other forms of life. Carbolic acid kills the staphylococcus wintering in the carbuncle in the professor's neck—the same chemical will kill the owner of the carbuncle if it is applied inside his neck and not outside of it.

Bichloride of mercury is a fine germicidal agent, and an equally efficient suicidal agent. Arsenic is rough on rats—and just as rough and rough in the same way on the germs of syphilis—and arsenic, you may remember, was the poisonous ingredient in Toffana water—that Saint-illumined beauty lotion, which when given internally—never failed in its sanctifying business.

And so it goes with most of our antiseptics and germicides—with the good they do in paralyzing the wicked germ—they interfere with normal living processes. It is a trite though often correct observation—that an operation may be successful though the patient passes on.

Equally correct is it to say that any vicious germ floating in the blood stream can be killed by introducing into the circulation the

appropriate poison—only that the germ's host will be quite as abruptly killed.

The ideal germicide is the one that kills invading germs with the least possible damage to the living tissue harboring the germ.

Of course we must remember also, whether we like arithmetic or not, that the dilution factor always enters into the question of germicides. Einstein's theory of relativity may be hitched to the stars, but it touches most every earthly problem too.

So with germicides—so with poisons. The ideal germicide might be defined as one which is not poisonous to animal cells in doses fatal to disease germs—one which is chemically compatible with tissue, one which has a high coefficient of penetration and one which stays just long enough in the tissue to perform its germ-killing ministry. Efforts have been made and are constantly being made to gather together as many as possible of these conditions into the conduct of new antiseptic chemicals.

Thus in the colorful antiseptic mercurochrome, the highly toxic qualities of mercury and its poor rate of dispersion, due to local coagulation, are compensated for by fluorescein, a dyestuff of high penetration and low toxicity.

A similar combination of iodine with fluorescein is available under the name of iodeosine (tetra-iodo-fluorescein $C_{20}H_8I_4O_5$). It has been used as an indicator, and possesses a color degree of dizzy red, not quite as spectacular, however, as that of mercurochrome. It has never been suggested as an antiseptic. It is unfortunately insoluble in water, although freely soluble in alcohol. A water-soluble sodium salt of this compound is known as erythrosine, and is a permissible coal tar color for camouflaging food. For all we know then, the bright red pop soda water of circus days may make a better gargle than a beverage.

Elemental iodine has a phenol coefficient of 4, that is, it is four times as effective as carbolic acid in squelching bad microbes. A 1-1000 solution is sufficient to kill most vegetative forms of bacteria including the staphylococcus, tubercle and typhoid bacillus. Dr. Lambert, reporting in the *Journal of Experimental Medicine* in 1916, states that of all the germicides then tested on germs in the presence of living tissue, a weak solution of iodine was the only one which would kill the bacteria without injuring the tissue cells.

The Research Department of the Philadelphia College of Pharmacy and Science has developed an isotonic solution of iodine which may to advantage replace the once famous Dakin's solution.

The commonest methods for exhibiting the medicinal as well as the germicidal effects of free iodine are in the form of alcoholic solutions, such as the tinctures of iodine and in aqueous solutions, such as Lugol's, where potassium iodide is used to increase the solubility of the iodine.

As a household antiseptic mild tincture of iodine will prove of real value. It combines the therapeutic qualities of iodine, as a counter-irritant and germicide with the local values of the solvent alcohol. It has a high degree of penetration in spite of its theoretical incompatibility with tissue.. It is still the surgeon's first choice in rendering sterile the site of operation. When that part of the anatomical landscape has been selected where the surgeon wishes to open his subject, the customary ante-slicing performance is a generous dousing with an alcoholic or an ethereal soap solution. After a water wash this is followed by a liberal allowance of tincture of iodine. Allowing a brief delay to permit the iodine to execute every single germ on location, the stain is cleared with alcohol, and the surgeon now gleefully goes to work. That is how much confidence most surgeons have in tincture of iodine.

As a local antiseptic application to fresh wounds and bruises—as a mild counter-irritant, it is not likely that any of the new dye compounds are superior to the century old alcoholic tincture of iodine. Of course it has some disadvantages—as have all blessings. Its dark disagreement with starched garments, its tendency to concentrate on long standing in unstoppered vials, its bad behavior when too tightly bandaged, and its poisonous nature when taken internally, are all against it.

Yet its stains with starch are readily removed with photographic hypo (sodium thiosulphate). Its tendency to concentrate on long standing in unstoppered vials is easily avoided by keeping it in stoppered vials, and not too long. Its bad behavior when a sore bathed with it is too tightly bandaged can be similarly avoided by withholding the bandage.

So too can its suicidal charms be mimimized. As a matter of fact one who chooses tincture of iodine as an easy means to an end, is unesthetic and unreasonable and indelicate, particularly if the last meal was tapioca or rice pudding. As a suicidal implement drowning

is quicker, cheaper and cleaner—whereas tincture of iodine is uncompromisingly slow and painful.

IVOR GRIFFITH.

METALLIC PARTICLES IN MANUFACTURED PRODUCTS, DETECTION AND IDENTIFICATION OF. H. C. Lockwood. (*Analyst,* 1934, *59,* 812, through *Quart. Jour. of Pharmacol. and Pharm.*). Before applying the tests, fat is removed by extracting three times with ether, and once with alcohol. Water-soluble substances are then removed by washing twice with water. The residue is dried and treated with the reagent on a microscope slide. The reagents are: N/10 silver nitrate; auric chloride, 2.5 gm. in 100 mils of water with 1.5 mils of nitric acid and 1.25 mils of hydrochloric acid; mercuric chloride solution; the acid fumes arising from a mixture of concentrated hydrochloric and nitric acids; and certain special reagents for individual confirmatory tests. Summary of results, observed through microscope:—*Iron.*—Detected by magnet. Bubbles of gas, and a haze with auric chloride. Blue patches with potassium ferrocyanide after exposure to acid fumes. *Nickel.*—Detected by magnet. Gold-coloured "tree" with auric chloride. Red colour after exposure in turn to acid fumes, ammonia fumes and dimethylglyoxime solution. *Zinc.*—Silver "tree" with N/10 silver nitrate. Hydrogen with auric chloride. Lead "tree" with solution of lead acetate. *Tin.*—Characteristic silver "tree" with N/10 silver nitrate. Purple growth with auric chloride. *Aluminium.*—Hydrogen with auric chloride, and with mercuric chloride. *Copper and Brass.*—Distinctive colour by reflected light. Silver "tree" with N/10 silver nitrate. Pink colour with potassium ferrocyanide, after sample has been exposed first to acid fumes. Yellow precipitate after exposure to acid fumes and then to potassium iodide solution. This yellow colour (iodine) becomes blue on addition of starch solution. *Lead.*—Silver "tree" with N/10 silver nitrate. Black "tree" with auric chloride. White deposit with mercuric chloride. After exposure to acid fumes, and addition of potassium iodide solution, a yellow colour appears, unaltered by starch solution. *Solder.*—Reactions of tin and lead, combined. *Mercury.*—Spheroidal globules which scatter droplets on pressure. "Tree" with silver nitrate, silver-coloured by reflected light. *Chromium.*—Bright green spots when exposed to acid fumes. No auric chloride reaction. Six typical photomicrographs are reproduced.

A. A.

ORIGINAL ARTICLE

BIO-CHEMISTRY OF AMYGDALIN*
(BITTER, CYANOGENETIC PRINCIPLE FROM BITTER ALMONDS)

By Arno Viehoever and Harry Mack

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*Presented before the American Association for the Advancement of Science, meeting at Pittsburgh, Pa.

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PART I

This detailed study of amygdalin is an elaboration of work started 100 years ago by the renowned chemists Liebig and Wöhler. It illustrates the importance of varied "hydrolysis" in nutrition and medication and demonstrates the usefulness of daphnia as test animals.

Introduction

Amygdalin is one of the first and best known glucosidal substances isolated from plant products. It yields on transformation (hydrolysis) in the test tube and obviously also in the animal body a series of decomposition products. Those obtained as a result of enzyme action have received greatest attention, those resulting from alkaline action least consideration. The full understanding of the bio-chemistry of amygdalin embraces not only the physiological effects of amygdalin itself, but also of all the derivatives formed in its transformation in the animal body. Their different chemical and —as we shall show—physiological character makes amygdalin and its split products some of the most interesting substances.

As this nitrogenous glucoside imparts a bitter taste to products containing it, and as it yields a striking flavor of bitter almond oil (benzaldehyde) as well as the toxic hydrocyanic acid—under certain conditions—it has excited much inquiry, both from a physiological and from an economical point of view. Quite a number of plants and plant products, used rather extensively as a food or feed, may yield the same poisonous acid. The safety from poisoning has therefore assumed importance with respect to human and animal welfare. It has been deemed expedient to determine the conditions which either prevent or effect the formation of hydrocyanic acid, and which influence the various complete or partial hydrolyses. We have consequently included in our scope the study of the products obtained as a result of enzymatic, acid—and alkaline hydrolysis. One or the other of these processes may take place in the alimentary tract as the acid stomach or the alkaline intestine.

PART II

Historical

1. a) *Amygdalin and Its Enzymatic Hydrolysis*

The presence of hydrocyanic acid in the aqueous distillate from bitter almonds was first observed by Böhm in the early part of the nineteenth century. The crystalline glucoside amygdalin, however, was isolated later in 1830 by Robiquet and Boutron (1), who in addition also indicated its relationship to the essence of bitter almond.

In 1837, Liebig and Wöhler (2) found that amygdalin was hydrolized by a nitrogenous enzyme associated with the glucoside in the almond. To this substance they gave the name emulsin.

It was Schiff (3), however, in 1870, who was first to suggest that the two glucose residues in the molecule of amygdalin were united as a biose, indicated as follows:

$$C_6H_5CH(CN).O.C_6H_{10}O_4.O.C_6H_{11}O_5$$

This view prevailed until Fischer (4) showed that amygdalin may be resolved, by an enzyme found in yeast extract, into a new substance with one glucose residue which he named mandelonitrile glucoside. He thereby came to the erroneous conclusion that amygdalin was a derivative, perhaps of maltose or of some other closely related diglucose. The assumption that amygdalin is a maltoside has been also advanced by Dunsten and Henry (5). Recent work, however, does not support this supposition because neither in its chemical properties nor in its behavior toward enzymes does it act as a maltoside.

b) *Acid Hydrolysis with Dilute and Concentrated Acids*

In 1856, Ludwig (6) demonstrated that hot dilute mineral acids hydrolyze amygdalin with the formation of products analogous to those obtained from enzymatic hydrolysis. These substances are benzaldehyde, hydrogen cyanide and glucose resulting in accordance with the following equation:

$$C_{20}H_{27}O_{11}N + H_2O = C_6H_5CHO + 2C_6H_{12}O_6 + HCN$$

When subjected to the hydrolytic action of concentrated hydrochloric acid, amygdalin yields l-mandelic acid as one of the final products which is directly related to Fischer's d-mandelonitrile glucoside. The intermediate products, moreover, are amygdalinic acid and ammonia. Courtauld and Caldwell (7) have shown, in their quantitative studies of the hydrolysis of amygdalin by acids that the first decomposition occurs at the biose junction, causing the removal of a molecule of glucose resulting in the production of mandelonitrile glucoside obtained by Fischer. Emulsin, however, hydrolyses amygdalin at both glucose junctions simultaneously, producing benzaldehyde cyanohydrin as an intermediate product.

Walter and Krieble (8) have indicated that concentrated sulphuric acid shows very little tendency toward attacking the nitrile group but does eliminate d-mandelonitrile. The biose junction, however, is the point most easily affected by this acid in all concentrations.

In their observations of the activity of acids upon amygdalin, Courtauld and Caldwell attempted to estimate the quantity of glucose formed by titrating neutral hydrolyzed samples with Pavy's solution.* They record that a green coloration was developed which interfered with the end point. They accordingly made the deduction that the unexpected reaction resulted "as a consequence of the amygdalin being hydrolyzed by the boiling alkali," but did not investigate the matter any further. Walker and Krieble, in a continuation of the observations upon acid hydrolysis, made the statement that laevo-amygdalin is rapidly converted, at ordinary temperatures by a small quantity of an alkaline solution, into a much more soluble substance which yields racemic mandelic acid and not l-mandelic acid with concentrated hydrochloric acid.

It is a recognized fact that prulaurasin is a racemic mixture of the two stereoisomeric B-glucosides of dextro and laevo mandelonitrile and is analogous to isoamygdalin which was first prepared by Walker and subsequently further studied by Dakin. It likewise yields inactive mandelic acid when hydrolyzed by acids. Indeed, more striking is the fact that prulaurasin can be prepared by the action of a slightly alkaline solution upon either or both dextro and laevo mandelonitrile glucosides. The simplest possible assumption that can be made regarding this transformation, therefore, is that it consists of the racemisation of the asymmetric carbon atom.

c) *Alkaline Hydrolysis*

Liebig and Wöhler, in their work entitled "The Synthesis of Amygdalinic Acid" (2), explained in more detail the results of the alkaline treatment of amygdalin, determined the chemical composition and constitution of amygdalinic acid but only a few of its properties.

Likewise Schiff (3) duplicated and extended Liebig and Wöhler's work by investigating the constitution of amygdalin and amygdalinic acid, prepared acyl and benzoyl derivatives but also failed to determine many of the physical constants of amygdalinic acid. In fact, the literature, as far as a thorough search shows, is with the exception, noted below, devoid of any constants other than several

*Pavy's solution is an ammoniacal Fehling's solution made by dissolving (a) 4.158 gm. of copper sulphate in enough water to make 500 ml., (b) 20.4 grams Rochelle salt, 20.4 grams potassium hydroxide, 300 cc. of 28 per cent. NH_4OH in enough water to make 500 ml. For use mix equal volumes of (a) and (b).

solubilities. Walker (9), however, does give the specific rotation of solutions of amygdalin, which have been hydrolyzed by $Ba(OH)_2$, incidental to his work upon the catalytic racemisation of amygdalin by alkali. His results showed that most probably racemisation does not occur, neither when the nitrile group is hydrolyzed to carboxyl nor when the diglucose radicle is replaced by the hydroxyl group, but on the contrary is effected without any apparent chemical reactivity when amygdalin is dissolved in dilute alkaline solution. He has found that the amygdalinic acid so produced is truly racemoid and optically inactive.

2. *Biochemistry*

The biochemic study of amygdalin in the past has been limited mostly to that of the physiological effects of amygdalin and hydrocyanic acid. Few data are available on the physiological function of ammonia, even less on that of benzaldehyde, none on the physiological effects of amygdalinic acid and l-mandelic acid.

III

Amygdalin

Occurrence

In general, amygdalin occurs as a crystalline substance in the kernels or seeds of certain rosaceae, as for instance peaches, cherries, plums, apples and particularly abundantly in bitter almonds. Its presence and the amount found in plants and plant products is recorded in Wehmer (11) and indicated in Table I. From many of these sources crystalline amygdalin can be isolated. Its amorphous isomers prunasin or prulaurasin are transition products and may be found in the growing tissues as young buds, leaves and branches, while the crystalline amygdalin, as the end product is stored in the seeds.

It may best be prepared as follows:

Preparation:

Bitter Almonds

(Shelled, Powdered)

Extract fatty matter and oil with Petr. ether. Boil fat free almond residue repeatedly with alcohol (95 per cent.). Filter and cool collected alcohol extracts. Recrystallize the precipitate amygdalin from hot alcohol.

Plant Name	Plant
Tridens flavus (L.) H.	Tops, ste
Agropyrum repens B.	Rhizome
Lasia Zollingeri Sch. Lasia heterophylla Sch.	Flower-c
Smilax ornata H.	Root
Cannabis sativa L.	Seed
Aquilegia vulgaris	Blooming
Spiraea sorbifolia L.	Herb and
Spiraea japonica L.	Leaves
Pygeum latifolium Mg.	Bark
Pygeum parviflorum T. et B.	Bark
Pygeum africanum H.	Bark
Crataegus oxyacantha L.	Seed
Crataegus oxyacantha	Fruit (see
5 Crataegus species	
Cydonia vulgaris Pers.	Seed
Cydonia japonica Pers.	Seed
Cydonia oblonga Mill.	Seed
Pirus malus L.	Seed
Pirus communis L. (1)	Seed (cul
Pirus communis L. (2)	Seed (wil (wood pea
Sorbus aucuparia L.	Seed
Prunus Amygdalus Stok.	Seed
Prunus Sweet variety	Seed
Prunus Bitter variety	Seed
Prunus Bitter variety, fresh	Seed
Prunus Persica (Peach) Sieb. et Zucc.	Seed
Prunus Armeniaca (Apricot) fresh	Seed
Prunus domestica (Prune) L.	Seed
Prunus species, different	Seed
Prunus sphaerocarpa Sw.	Seed
Prunus avium L.	Seed
Prunus cerasus L.	Seed
Prunus virginiana M.	Seed
Prunus Mahalab L.	Seed
Prunus Laurocerasus L.	Seed
Prunus Padus L.	Seed
Gymnema Latifolium Willd.	Leaves

TABLE I

DISTRIBUTION OF AMYGDALIN AND EMULSIN

	Plant Part	Plant Family	Nature of Glucoside	Amount Per Cent.	Nature of Enzyme	Author	Journal	Volume	Pa
	Tops, stems, leaves	Gramineae	No Amygdalin (?)		Emulsin (?) Splitting Amygdalin	Viehoever, Johns and Alsberg	*J. Biol. Chem.*	25	1
	Rhizome	Gramineae	No Amygdalin (?)		Emulsin (?) Splitting Amygdalin	Rosenthaler (1)	*Arch. Pharm.*	251	5
	Flower-cone	Araceae	No Amygdalin		(?)	Greshoff (1)	*Ber.*	23	353
	Root	Liliaceae	No Amygdalin (?)		Emulsin (?) Splitting Amygdalin	Power and Salway	*J. Chem. Soc.*	105	20
	Seed	Cannabinoideae			Emulsin (?) Splitting Amygdalin	Rosenthaler (1)	—	—	—
	Blooming plant	Ranunculaccae	Amygdalin-like			Jorissen and Hairs	*Pharm. Post.*	24	65
	Herb and Flowers	Rosaceae	Amygdalin (?)			Wicke	*Ann. Chem.*	83	175
	Leaves	Rosaceae	Amygdalin (?)			Greshoff (2)	*Ber.*	23	354
	Bark	Rosaceae	Amygdalin			Greshoff	*Ber.*	23	354
B.	Bark	Rosaceae	Amygdalin			Greshoff	*Ber.*	23	354
	Bark	Rosaceae	Amygdalin			Greshoff	*Arch. Pharm.*	244	398
	Seed	Rosaceae	Amygdalin			Wicke	*Annal. Chem.*	79	79
	Fruit (seed)	Rosaceae	—		Emulsin	Lehmann	*Pharm. Zeitschr.*	23	
		Rosaceae	Amygdalin		(?)	Brunswik (1)	*Oestr. Bot. Zeitschr.*		Nos. 5 69
	Seed	Rosaceae	Amygdalin		Emulsin	Rosenthaler (2)	*Arch. Pharm.*	250	298
	Seed	Rosaceae	Amygdalin		Emulsin	Stockmann	*Tr. N. J.*	14.1	240
	Seed	Rosaceae	Amygdalin	.53		Brunswik (1)	—	—	—
	Seed	Rosaceae		.6					
	Seed (cultivated)	Rosaceae	No Amygdalin		Emulsin (traces)	Rosenthaler (2)	—	—	—
	Seed (wild) (wood pears)	Rosaceae	Amygdalin trace (?)			Huber	*Landw. Uers. St.*	75	445
	Seed	Rosaceae	Amygdalin		Emulsin	Lutz	*Repert de Pharm.*		312
	Seed	Rosaceae				Rosenthaler (3)	*Ber. Pharm. Ges.*	32	237
	Seed	Rosaceae	Amygdalin	0-0.1		Rosenthaler (3)	*Ber. Pharm. Ges.*	32	237
	Seed	Rosaceae	Amygdalin	+.0(2.0-8.0) 3.2-4.3	Emulsin	Rosenthaler (3)	*Ber. Pharm. Ges.*	32	237
	Seed	Rosaceae	Amygdalin	3.3-4.0		Brunswik (1)	—	—	—
	Seed	Rosaceae	Amygdalin	2.0-2.3 3.6 av. (0.-6.5)		Rosenthaler (2)	—	—	—
	Seed	Rosaceae	Amygdalin	(0.0-8.43) 3.0 av.	Emulsin	Rosenthaler (2)	—	—	—
	Seed	Rosaceae	Amygdalin	.96		Rosenthaler (2)			
	Seed	Rosaceae	Amygdalin	1.0		Alpers	*Z. Unt. Nahr. Gen.*	34	433
				1.82		Kassner and Eckelmann	*Arch. Pharm.*	252	402
	Seed	Rosaceae	Amygdalin	.91		Peckolt	*Ber. Pharm. Ges.*	20	594
	Seed	Rosaceae	Amygdalin	.82; 2.0		Rosenthaler (2)	—	—	—
						Brunswik (1)	—	—	—
	Seed	Rosaceae	Amygdalin		Emulsin (?)	Rosenthaler (2)	—	—	—
	Seed	Rosaceae	L-Amygdalin			Rosenthaler (4)	*Schn. Ap. Ztg.*	60	477
	Seed	Rosaceae	Amygdalin			Wicke	*Ann. Chem.*	81	243
	Seed	Rosaceae	Amygdalin		Emulsin Amygdalase	Bridel	*J. Pharm. Chim.*	12	249
	Seed	Rosaceae	Amygdalin	1.5	Emulsin (?)	Heumann	*Repert. Pharm.*	23	360
	Leaves	Asclepiadaceae	Amygdalin	Abundant	No Emulsin	Greshoff (2)			

I

AMYGDALIN AND EMULSIN

Nature of Enzyme	Author	Journal	Volume	Page	Year
Emulsin (?) Splitting Amygdalin	Viehoever, Johns and Alsberg	*J. Biol. Chem.*	25	141	1916
Emulsin (?) Splitting Amygdalin	Rosenthaler (1)	*Arch. Pharm.*	251	56	1913
(?)	Greshoff (1)	*Ber.*	23	3537	1890
Emulsin (?) Splitting Amygdalin	Power and Salway	*J. Chem. Soc.*	105	201	1914
Emulsin (?) Splitting Amygdalin	Rosenthaler (1)	—	—	—	—
	Jorissen and Hairs	*Pharm. Post.*	24	659	1891
	Wicke	*Ann. Chem.*	83	175	1852
	Greshoff (2)	*Ber.*	23	3548	1890
	Greshoff	*Ber.*	23	3548	1890
	Greshoff	*Ber.*	23	3548	1890
	Greshoff	*Arch. Pharm.*	244	398	1906
	Wicke	*Annal. Chem.*	79	79	1851
Emulsin	Lehmann	*Pharm. Zeitschr.*	23		1885
(?)	Brunswik (1)	*Oestr. Bot. Zeitschr.*		Nos. 58, 69	1923
Emulsin	Rosenthaler (2)	*Arch. Pharm.*	250	298	1912
Emulsin	Stockmann	*Tr. N. J.*	14.1	240	—
	Brunswik (1)	—	—	—	—
Emulsin (traces)	Rosenthaler (2)	—	—	—	—
	Huber	*Landw. Uers. St.*	75	445	1911
Emulsin	Lutz	*Repert de Pharm.*		312	1897
	Rosenthaler (3)	*Ber. Pharm. Ges.*	32	237	1922
	Rosenthaler (3)	*Ber. Pharm. Ges.*	32	237	1922
Emulsin	Rosenthaler (3)	*Ber. Pharm. Ges.*	32	237	1922
	Brunswik (1)	—	—	—	—
	Rosenthaler (2)	—	—	—	—
Emulsin	Rosenthaler (2)	—	—	—	—
	Rosenthaler (2)				
	Alpers	*Z. Unt. Nahr. Gen.*	34	433	1917
	Kassner and Eckelmann	*Arch. Pharm.*	252	402	1914
	Peckolt	*Ber. Pharm. Ges.*	20	594	1910
	Rosenthaler (2)	—	—	—	—
	Brunswik (1)	—	—	—	—
Emulsin (?)	Rosenthaler (2)	—	—	—	—
	Rosenthaler (4)	*Schn. Ap. Ztg.*	60	477	1922
	Wicke	*Ann. Chem.*	81	243	1852
Emulsin Amygdalase	Bridel	*J. Pharm. Chim.*	12	249	1915
Emulsin (?)	Heumann	*Repert. Pharm.*	23	360	
No Emulsin	Greshoff (2)				

Physical Properties

Amygdalin crystallizes in rhombic columns from water. It melts at 200 degrees C., after loss of water of crystallization. It is soluble in all proportions in boiling water, and insoluble in ether.

Soluble in 12 pts. water of 8-12 degrees C.
Soluble in 904 pts. alcohol (94 per cent.) 8 degrees C. at boiling.
Soluble in 148 pts. alcohol (47 per cent.) 8 degrees C. at boiling.
Soluble in 11 pts. alcohol (94 per cent.) 8 degrees C. at boiling.
Soluble in 12 pts. alcohol (47 per cent.) 8 degrees C. at boiling.

Amygdalin is l-rotatory. Its specific rotatory power $[\alpha]$ °d is $-38.47°$.

Chemical Properties

It is an α and β diglucoside of benzaldehyde cyanohydrin. Upon addition of the enzyme emulsin, or an aqueous extract of sweet almonds, containing it, amygdalin is decomposed in benzaldehyde, hydrocyanic acid and glucose. $C_{20}H_{27}NO_{11} + 2H_2O \longrightarrow HCN + C_7H_6O + 2C_6H_{12}O_6$; 1 equivalent of amygdalin or 457 parts yield 1 equivalent of hydrocyanic acid or twenty-seven parts (100 parts of amygdalin give a theoretical yield of 5.90 parts of hydrocyanic acid). Seventeen grams of amygdalin, according to Liebig and Wöhler, yield one gram of hydrocyanic acid (that is 5.7 per cent.) and eight (four, calc.?) grams of benzaldehyde (or oil of bitter almond). Oxidation of amygdalin with manganese dioxide and dil. sulphuric acid causes formation of carbon dioxide, ammonia, formic acid and benzaldehyde. Oxidation with potassium permanganate causes formation of potassium benzoate and potassium cyanate. Boiling with alkali (KOH or $Ba(OH)_2$) yields ammonia and amygdalinic acid. Boiling with acid (HCl conc.) causes decomposition into l-mandelic acid, glucose and ammonium chloride.

Physiological Properties

Oral Administration: According to Reid Hunt (1a) amygdalin is only poisonous for higher animals to the extent that it is decomposed in the body with the formation of HCN. This decomposition is effected either through enzymes introduced with the food, or already present in the body or possibly through action of bacteria (10).

Mollusks: Amygdalin fed to Helix pomatia and Aplysia punctata, without emulsin, caused death, an odor of HCN being noted in the body.

Dogs: Death rarely has been reported, even after oral administration of amygdalin in large amounts. Toxic symptoms of HCN poisoning were observed, after 3 to 5 gm. amygdalin were given with ordinary food or without any food. Diarrhoea was observed.

Dogs and Cats: As a prominent effect, the feces have the odor of benzaldehyde.

Rabbits: Large amounts of amygdalin introduced in the intestine sometimes cause death.

Rabbits and Guinea Pigs: 0.4-0.6 g. per os proved fatal in one to two hours.

Herbivores: Possibly fatal, on account of hydrolysis through decaying processes in the intestine.

Rabbits and Guinea Pigs: Intraperitoneal injection of amygdalin in doses of 1 g. pro kilo had no effect. 0.14-0.25 gm. amygdalin pro kilo injected in the fassion usually are fatal—often in only a few minutes; the amygdalin splitting ferment diffuses probably into the peritoneal cavity of the small intestine.

Rabbits and Dogs: No toxic symptoms were observed upon injection subcutaneously and intravenously of relatively large doses (0.5 g. or more per kilo).

Daphnia: The use of this transparent crustacean for physiological experiments has been suggested by the senior author (2a-6a). Amygdalin, in agreement with the results obtained for higher vertebrate animals, is evidently only poisonous for daphnia to the extent to which it is decomposed in the body, forming hydrocyanic acid. It had the laxative and paralyzing action attributed to amygdalin—and its split product, hydrocyanic acid, when released in small amounts upon higher animals. Of sixteen daphnia introduced into a 2 per cent. solution of amygdalin, one was down and practically dead after four hours; two daphnia were dead after nine hours; all others, showing symptoms of narcosis, were still alive after sixteen hours.

In a 4 per cent., practically saturated, solution of amygdalin six of sixteen animals were dead after sixteen hours and the remainder died within eight more hours. Evidently amygdalin was very slowly hydrolyzed by the digestive enzymes of the body.

IV

PRODUCTS OF ENZYMATIC AND DILUTE ACID HYDROLYSIS

1. Hydrocyanic Acid

Occurrence

First prepared by Scheele (1782), hydrocyanic acid was isolated by Vanquelin (1803) from plants. Three hundred and sixty species of 148 genera and forty-one families have been reported to contain or yield hydrocyanic acid, either free or, as mostly, in the form of glucosides. At least ninety-six plants yield both benzaldehyde and hydrocyanic acid, the enzymatic split products of amygdalin, prunasin, prulaurasin and other cyanogenetic glucosides, mentioned later.

Preparation

For the preparation of benzaldehyde free HCN proceed as follows:

Heat potassium ferrocyanide (coarsely grd.) 10 pts., with dil. sulphuric acid (conc. 7 pts. + H_2O 20-30 pts). Distill.

Physical Properties

HCN is a colorless, tasteless liquid with a faint odor of peaches. It boils at 28 degrees C. and solidifies at 15 degrees C. It burns with a violet flame. It is miscible with water, alcohol and ether in all proportions. As a very weak acid it is freed even by carbon dioxide from its solutions.

Chemical Properties

The acid is only stable in pure form or in the presence of dilute acids. Concentrated HCl forms formamid and, upon further hydrolysis, formic acid. In the form of cyanogenetic glucosides HCN is very stable, although they are readily hydrolyzed by either enzymes or dilute acids, as we have mentioned. Cyanhydrines are intermediary products of both synthesis and decomposition.

Physiological Properties

The toxic and lethal effects of hydrocyanic acid are enumerated in Hunt's monograph (7a). The data given on Table II for the effect on vertebrates are taken from that survey.

EFFECT OF HYDROCYANIC ACID

ON VERTEBRATES

	Wgt.	Oral Admi.	L. D.	Pro Kilo.	Subcutan. Ad.	L. D.	Pro Kilo.	Intravenous	L. D.	Per Kilo.	Remarks
Man		Oral (?)	appr. 0.5 mgr.	"			"				
Monkey					Subcutan.	appr. 3.0 mgr.	"				
Dog		"	4 mgr.	"	"	1.1–1.7 mgr.	"	"	0.54 mgr.	"	I. Trij. Death in 17 min.
Cat					"	1.1–2.16 mgr.	"	"	1.5–2 mgr.	"	
Rabbit		"	4 mgr.	"	"	1.1–3.0 mgr.	"	"	0.33–0.4 mgr.	"	
Sheep		"	6.28 mgr.	"							
Rat					"	10.15 mgr.	"	"	2.5 mgr.	"	
Mouse					"	3.0–3.4 mgr. 4.39–5 mgr. 12.8 mgr.	" " "	"	2.5 mgr. (KCN)	"	
Guinea Pig					"	6.7–8.1 mgr. 5.0 mgr.	" "				
Pigeon					"	2.15 mgr. 2.4 mgr.	" "				
Porcupine		"			"	4.8 mgr. 5.85 mgr.	" "				
Fish (Cyprinus Gibelis)		Immersion	1:25000								Death in several hours
Frog					" (lymph sac)	0.06 mgr.	"				
Tadpoles		"	1:10,000 NaCN 1:100,000 KCN								Death in 15 min. Death in 3 hrs.

The action of hydrocyanic acid upon invertebrates has been studied to a considerable extent, particularly in connection with the use of this poisonous gas as an insecticide. However, its action on high as well as on lower forms, including even one-celled protozoa, has established the conviction that hydrocyanic acid is a general blood and plasma poison.

More rapid conversion of amygdalin to poisonous hydrocyanic acid in the alimentary tract by means of digestive enzymes is achieved by the enzyme emulsin. The concentration of this enzyme determined the amount and speed with which the transformation took place. Emulsin added in amounts of 0.1 per cent. caused sufficient formation of hydrocyanic acid (and benzaldehyde) to kill daphnia (fourteen animals) within sixty to seventy minutes; a new lot (fourteen) of daphnia, then placed into the very same solution, died within thirty to fifty seconds, evidently due to the comparatively large amount of hydrocyanic accumulated. Adding 1 per cent. emulsin to the 2 per cent. amygdalin solution caused the death of all animals within five to seven minutes, showing successively the aggravated symptoms characteristic of poisoning by hydrocyanic acid.

2. Benzaldehyde

(Bitter Almond Oil)

Historical

First obtained by Martrès (1819) as a volatile oil from bitter almonds, Wöhler and Liebig (1837) recognized its formation as due to the enzymatic decomposition of amygdalin in bitter almonds into benzaldehyde and hydrocyanic acid.

Occurrence

Benzaldehyde, found as such in minute amount in Sumatra benzoe, occurs mainly in the volatile constituents obtained from plants and plant products containing glucosides, splitting off hydrocyanic acid and benzaldehyde. Such glucosides, in addition to amygdalin, are samburnigrin in Sambucus nigra and pyramidalis; amygdonitrilglucoside in Cerasus padus Delarb and Prunus serotina Ehr.; prulaurasin in Cotoneaster microphylla Wall and Prunus laurocerasus; vicianin in Vicia augustifolia.

Physical Properties

Benzaldehyde is a colorless liquid with the smell of bitter almond oil, with burning, aromatic taste, strongly light refractive, easily inflammable, producing a glaring, charring flame. The specific gravity at 15 degrees C. is 1.05. It is only little soluble in water (approx. 0.3 per cent.) but miscible in all proportions with alcohol, ether, fatty and ethereal oils. It is volatile with steam; boiling at 179 degrees to 180 degrees C.

Chemical Properties

Benzaldehyde is readily oxidized through oxygen or oxidants or even on exposure to air to benzoic acid; it is readily reduced with nacent hydrogen to benzylalcohol. It is further characterized by salt formation, forming crystalline addition compounds with alkali bisulphites; with strong ammonia it forms the non-basic hydrobenzamide, colorless crystals, melting at 110 degrees C., insoluble in water. With concentr. sulphuric acid benzaldehyde gives a red color (orange red to brown) followed by charring on heating.

Physiological Properties

Benzaldehyde is completely harmless, according to Wöhler and Frerichs (17a), Schimmel and Co. (8a) or only a slightly poisonous substance, Macht (16a), when chemically pure. According to others it causes disturbances in the metabolism and central cramps (Kunkel) (9a) (appearing much like epileptic cramps) (Beckurts, Jabresber f. Pharmazie 1893) (10a), when given in large amounts (Lewin) (11a). Upon prolonged contact with blood (Lewin 1898) or with 1 per cent. blood solution (Kobert) (12a) benzaldehyde forms methemoglobin. It is a local anesthetic (16a).

The experimental work, as reported in literature (13a-15a), is recorded on the following tables. Our own work with vertebrates and invertebrates (daphnia) is also tabulated. It is obvious from these results that the physiological action and toxic effect becomes quickly evident, if rapid and substantial oxidation to benzoic (hippuric and cinnamic?) acids cannot take place (14a, 17a). As benzaldehyde dissolves cholesterol and thus cell lipoids, it causes toxic effects of narcosis, spasmic and depressed breathing, and even death, when administered orally or subcutaneously in amounts of 3.5 cc. or 3 cc. respectively per kg. of bodyweight. In 0.4 to 0.87 per cent. benzaldehyde culture water solution, paralysis, followed by certain death, occurs in five to twenty seconds; in a 0.017 per cent. solution, within one to two hours.

EFFECT OF BENZALDEHYDE
ON VERTEBRATES

Animal	Weight	Form of Application	Dosage	Symptoms	Author	Remarks
Frog		Subcutaneous	0.1-0.3 cc. undiluted; if not too great	Complete paralysis, no clonic cramps. Recovery.	Baglioni	
Frog		Subcutaneous	2% aq. sol. 1 cc.	After prolonged time—weak paralysis. No motoric disturbance.	Baglioni	
Frog		Transfusion	1 cc. added to 400 cc. ordinary blood mixture	After 5 minutes: Marked paralysis, without preceding excitement. After 15 minutes: Paralysis increased, but incomplete; very weak. Response to strong mechanical irritation.	Baglioni	
		Transfusion	Pure blood mixture	After 5-10 minutes: Complete recovery. No clonic cramps. Normal spontaneous movements. Normal reflectory movements.	Baglioni	
Dog		Orally (in capsules)	Daily up to 10 gr. in 3 portions totally 65.8 gr.	No physiological symptoms reported. 1. Benzoic acid. 2. Hippuric acid, large amounts. 3. Cinnamic acid (traces?). 4. Benzamid (4% of amt. of benzaldehyde) found in urine.	Cohn	Food: Milk and Bread

EFFECT OF BENZALDEHYDE—ON VERTEBRATES—(Continued)

Animal	Weight	Form of Application	Dosage	Symptoms	Author	Remarks
Dog		Orally (into stomach) Ammonium benzoate	Daily 10 gr. Totally 30 gr.	No physiol. symptoms reported. Benzamid 0.1 gr. Hippuric acid (mainly) found in urine and benzoic acid.	Cohn	
Rabbit		Subcutan. Addition of Sodium Carbonate or Sodium Acetate →	Daily 2.8 gr. in 2 portions for 3 days	Stood it very badly, died after 2-4 days. Symptoms: weakening, paralysis, tremor, diarrhea, nephritis, one with hemoglobinuric. No noticeable change in physiol. response. 1. Benzaldehyde—traces. 2. Benzoic acid. 3. Hippuric acid (mainly). 4. Cinnamic acid (small amount? found in urine).	Cohn	
Dog (female 9 yrs.)	8.8 kilograms	Orally (capsules)	4.40 cc.	Became almost immediately drowsy and sick, showed weakness, vomited after 30 minutes. Recovered.	Viehoever and Mack	Administered 2 hours after feeding.

EFFECT OF BENZALDEHYDE—ON VERTEBRATES—(Continued)

Animal	Weight	Form of Application	Dosage	Symptoms	Author	Remarks
Rat (male)	210 gm.	Subcutaneous (intraperiton.)	0.45 cc.	After 1 minute: Spasmic breathing. Respiratory rate slowed from 204 to 182 per minute. After 30 minutes: Continued spasmic breathing. Sensitivity somewhat reduced. After 40 minutes: Respiratory rate slowed down to 60 per minute. After 60 minutes: Respiratory rate increased to 112 per minute. After 70 minutes: Somewhat more responsive. Still under normal. After 80 minutes: No marked change. After 20 hours: Complete recovery. (Eyes never closed during first hour and 20 minutes.)	Viehoever and Mack	Administered 5 hours after feeding. Water available

EFFECT OF BENZALDEHYDE—ON VERTEBRATES—(Continued)

Animal	Weight	Form of Application	Dosage	Symptoms	Author	Remarks
Rat (female)	192 gm.	Subcut. (intraperiton.)	0.41 cc.	After 13 minutes: Remains stationary. After 15 minutes: Trembles 2-10 seconds apart. After 32 minutes: Respiration rate slowed from 180 to 90 per minute. Eyes are not closed. After 27 minutes: Animal shows cramp-like contraction in anterior pit of body. After 83-100 minutes: Animal urinated bloody urine, showing hemoglobinuria and nephritis (albuminurea). After 120 minutes: Respiration rate 88 per minute. Subsequent recovery.	Viehoever and Mack	Administered 4 hours after feeding. —— Water available.

EFFECT OF BENZALDEHYDE—ON VERTEBRATES—(Continued)

Animal	Weight	Form of Application	Dosage	Symptoms	Author	Remarks
Rat (female)	172 gm.	Subcut. (intraperitoneally)	0.5 cc.	After 3-4 minutes: Spasmic breathing. Respiratory rate slowed from 182 to 60 per minute. Eyes not closed. After 4-6 minutes: Front leg stretched out sideways. Insensitive to severe mechanical irritation. After 8 minutes: Muscles relaxed—breathing rate rapidly slowed down. Limbs relaxed. After 9 minutes: Death. Post mortem: Lung—partly collapsed. Stomach—gas. Intestine—gas. Liver—somewhat violet color.	Viehoever and Mack	Administered 4½ hours after feeding.

EFFECT OF BENZALDEHYDE—ON VERTEBRATES—(Continued)

Animal	Weight	Form of Application	Dosage	Symptoms	Author	Remarks
Rat (male)	220 gm.	Subcut. (intraperiton.)	0.5 cc.	After 3 minutes: Evacuation (solid excr.). After 4 minutes: Breathing slightly slowed from 190 to 180 per minute. Trembling of head accentuated by spasmic breathing; this becoming more labored. Animal becoming stationary, motionless. After 7-9 minutes: Breathing rate slowed down to 124 from 132 per minute. After 15 minutes: Breathing very labored; spasmodic and deep. After 19 minutes: Breathing rate 128 per minute. After 25 minutes: Breathing rate 116-112 per minute. Weak response to irritation. After 30 minutes: Breathing rate 120 per minute. Animal lies on abdomen, eyes open, breathing deep labored. After 52 minutes: Breathing rate 112 per minute. Within 20 hours: Animal died (during night).	Viehoever and Mack	Administered 6½ hours after feeding. — Water available.

EFFECT OF BENZALDEHYDE—ON VERTEBRATES—(Continued)

Animal	Weight	Form of Application	Dosage	Symptoms	Author	Remarks
Rat (female)	172 gm.	Orally	0.57 cc.	After 5 minutes: Respiratory rate slowed from 180 to 120 per minute. After 30 minutes: Spasmic breathing. Exaggerated and unusual shaking of head. Animal unable to hold eyes open. Mostly in corner holding head down. After 90 minutes: Animal more lively, eyes still partly closed. After 135 minutes: Sudden body reactions, like contractions, suggest cholic-like pains. No further marked changes. Within 20 hours: Recovery. (Next day.)	Viehoever and Mack	Administered 5 hours after feeding. —— Water available.

EFFECT OF BENZALDEHYDE—ON VERTEBRATES—(Continued)

Animal	Weight	Form of Application	Dosage	Symptoms	Author	Remarks
Rat (male)	257 gm.	Orally	Appr. 0.5 cc.	Immediately: Animal moves in cage back and forth, restless, shows irritation on mouth, where benzaldehyde spilled. After 5 minutes: Closes eyes for about 10 seconds and opens eyes again. After 10 minutes: Animal stationary in one corner. After 35 minutes: Still responses to irritation. After 45 minutes: Hardly responses. After 55 minutes: Lying in corner somewhat on side, crouched together, eyes closed. After 60 minutes: Eyes half closed; animal obviously weakened. Respiratory rate 80 per minute. After 65 minutes: Getting up out of corner. More lively. After 70 minutes: Back in corner, turning away from light. Within 18 hours: Animal lively, normal, eats food readily.	Vichoever and Mack	Administered 6 hours after feeding. Water available

EFFECT OF BENZALDEHYDE—ON VERTEBRATES—(Continued)

Animal	Weight	Form of Application	Dosage	Symptoms	Author	Remarks
Rat (female)	210 gm.	Orally	0.7 cc.	After 1-2 minutes: Closing eyes temporarily. After 5 minutes: Breathing rate slowed from 238 per minute to 78 per minute. After 5 minutes: Eyes kept mostly closed; when opened only open for a few second's interval. After 15 minutes: Respiratory rate 105 per minute. Excrements were released. Eagle-spread position. After 25 minutes: Somewhat more lively. After 60 minutes: Breathing irregular. Spasmic—slight tremor. After 65 minutes. Respiration rate 102 per minute. Breathing shallow. Eyes open. After 156 minutes. Animal lying very quietly. Respiration rate 74 per minute. Animal died probably of asphyxiation. Post mortem: Lung completely collapsed. Much gas in stomach, ¾ filled with gas. In small and large intestine evidence of gas. Liver somewhat violet color.	Viehoever and Mack	Had no access to water or food after being fed normally 4 hrs. before adm. of benzaldehyde

EFFECT OF BENZALDEHYDE
ON DAPHNIA

Daphnia female	17 days egg free	Orally (in culture water)	t. 21.5 C. appr.	0.78%	After 5 seconds: Two animals dead (motionless).
Daphnia female	17 days egg free	Orally (in culture water)	t. 21.5 C. appr.	0.39%	After 12 seconds: No visible life activity. After 20 seconds: No visible life activity.
Daphnia female	16 days with eggs	Orally (in culture water)	t. 21.5 C. appr.	0.4%	Practically instantaneous death.
Daphnia female	17 days old egg free	Orally	t. 21.5 C.	0.156%	After 56 seconds down—Trembling of antennae. After 77 seconds down—Trembling of antennae. After 3 minutes: both down lying on side, whole body frame in tremor. After 14½ minutes: Digestive canal moves intermittently. Breathing, legs move faintly. Heart beat—slow-regular, twitching of antennae muscles. Down—in tube. Breathing irregular, faint. Constriction of lower intestines.

EFFECT OF BENZALDEHYDE—ON DAPHNIA—(Continued)

Daphnia	17 days old egg free	Orally (in culture water)	t. 21.5 C.	0.078%	Almost immediately: Greatly increased locomotory action. Two animals swim constantly with rapid motions. After 1 minute 53½ seconds: Swimming upside down. After 4½ minutes: Considerable trembling. After 8½ minutes: Swimming vigorously near surface, somewhat like in strychnine action. After 17 minutes: Movement no longer so violent. Breathing slowed down, trembling of body. One—more or less down. One—on top. After 30 minutes: Still active; but weakened (handicapped).
Daphnia	16 days old	Orally	t. 21.5 C.	0.01%	After 4 minutes: Paralysing—trembling. After 11 minutes: Paralysing of breathing organs. After 2 hours: Still living. After 2 hours, 19 minutes: Heartbeat irregular, appr. 60 per minute. Breathing practically paralysed; on bottom.

EFFECT OF BENZALDEHYDE—ON DAPHNIA—(Continued)

Daphnia	16 days old	Orally	t. 21.5 C.	0.008%	After 5 minutes: Heart faintly beating. After 45 minutes: After 62 minutes: Heart action—out. Washing out with culture water. Partial recovery. After 4 hours: Addition of traces of NH_3, remarkable stimulation.
Daphnia	16 days old	Orally	t. 21.5 C.	0.008%	After 3¾ hours: Breathing O.K. Heartbeat—shallow. After 24 hours: Still active—though weakened.
Daphnia	16 days old	Orally	t. 21.5 C.	.004%	After 2 hours: Still living on bottom. After 3 hours: Partial paralysis. Still breathing. After 4 hours: More or less on bottom and intermittent activity. After 24 hours: Still living.

EFFECT OF BENZALDEHYDE ON DAPHNIA
(0.01% SOLUTION)

No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10
After 35 sec. Animal down	After 45 sec. Animal down	After 20 sec. Animal down	After 30 sec. Animal down	After 40 sec. Animal down	After 45 sec. Animal down	After 35 sec. Animal down	After 40 sec. Animal down	After 35 sec. Animal down	After 40 sec. Animal down
After 1-2 min. Intestines move freely	After 1-2 min. Intestines move freely Breathing paralysed	After 1-2 min. Breathing paralysed Trembling	After 1-2 min. Breathing paralysed	After 45 sec. Breathing paralysed	After 55 sec. Breathing paralysed	Breathing practically paralysed	Breathing practically paralysed	Trembling	Breathing practically gone. Trembling
After 2 min. swimming legs extended	After 2 min. swimming legs extended	After 2 min. swimming legs extended	After 2 min. swimming legs extended	After 2 min. Tremors swimming legs extended	After 2 min. Tremors swimming legs extended	After 2 min. Slight tremors	Slight tremors		
After 3 min. No laxative action	After 3 min. slight evacuation	After 3 min. spasmic movement	After 3 min. Spasmic movement	After 4 min. Motionless	After 4 min. Motionless	After 3½ min. Eye seemingly motionless	After 3½ min. Eye motionless	After 8 min. Breathing slight. Eye motionless	
After 11 min. paralysis of eye. Heartbeat 68 per min.	After 11 min. Paralysis of eye		After 18 min. no heart action	After 13 min. Heartbeat 84 per min.	After 13 min. Heartbeat 20 per min. Breathing intermittent	After 14½ min. Heartbeat 80 per min.	After 14½ min. Heartbeat quivers. No breathing	After 15 min. Heartbeat 144 per min.	After 16 min. Heartbeat 120 per min.
	After 22 min. Heartbeat 92 per min.	After 27½ min. no movement of heart						After 28½ m. Breathing very feeble	After 28½ m. breathing very feeble
	After 55½ min. no heartbeat; no breathing	After 50 min. Heart quivers No heartbeat No breathing Intestines extended	After 50 min. no heart action. Intestines extended	After 46 min. Animal practically dead No twitching of legs. No breathing. No heart action	After 46 min. Animal practically dead No twitching of legs. No breathing. No heart action			Heartbeat 84 per min.	No heartaction
Within 110 min. Dead	Within 110 min. Dead	Within 124 min. Dead	Within 124 min. Dead	Within 98 min. Dead	Within 98 min. Dead	Within 78 min. Dead	Within 78 min. Dead	Within 70 min. Dead	Within 70 min. Dead

DE ON DAPHNIA
ON)

No. 6	No. 7	No. 8	No. 9	No. 10
r 45 sec. mal down	After 35 sec. Animal down	After 40 sec. Animal down	After 35 sec. Animal down	After 40 sec. Animal down
r 55 sec. thing lysed	Breathing practically paralysed	Breathing practically paralysed	Trembling	Breathing practically gone. Trembling
r 2 min. nors mming extended	After 2 min. Slight tremors	Slight tremors		
r 4 min. ionless	After 3½ min. Eye seemingly motionless	After 3½ min. Eye motionless	After 8 min. Breathing slight. Eye motionless	
r 13 min. rtbeat er min. thing rmittent	After 14½ min. Heartbeat 80 per min.	After 14½ min. Heartbeat quivers. No breathing	After 15 min. Heartbeat 144 per min.	After 16 min. Heartbeat 120 per min.
			After 28½ m. Breathing very feeble	After 28½ m. breathing very feeble
r 46 min. mal practly dead twitching legs. No thing. No t action			Heartbeat 84 per min.	No heart-action
nin 98 Dead	Within 78 min. Dead	Within 78 min. Dead	Within 70 min. Dead	Within 70 min. Dead

PART V

A. ALKALINE HYDROLYSIS, GENERAL AND EXPERIMENTAL

Alkaline Hydrolysis

General process with $Ba(OH)_2$ and KOH.

To verify the observations cited in literature, preliminary test tube experiments were conducted upon alkaline hydrolysis. Employing first approximately 0.1 gm. of amygdalin dissolved in 10 ml. of a filtered saturated aqueous solution of $Ba(OH)_2$ and then 0.1 gm. of amygdalin dissolved in 10 ml. of approximately N/2 KOH without any apparent chemical decomposition, the solutions were heated slowly at first, then rapidly increasing the temperature until the boiling point was attained. An appreciable odor of ammonia was detected issuing from the KOH solution at approximately 70 degrees C., whereas the evolution of the gas from the $Ba(OH)_2$ solution was not noticeable until 90 degrees had been reached. Total chemical transformation at boiling to a clear solution within twenty minutes was possible in the former case but in the latter solution decomposition occurred within one-half hour with a turbidity of $BaCO_3$.

Determination of the evolved ammonia.

The results suggested at once the possibility of applying this procedure to determine the purity of the amygdalin, as well as the quantity of amygdalinic acid produced provided the degree of hydration was known. Accordingly, a modified Kjeldahl method was applied, in which the evolved ammonia from a known weight of sample was collected in N/2 H_2SO_4, and the excess of acid titrated with N/2 NaOH, with methyl red as indicator. Based upon the dihydrate, the following results have been tabulated.

TABLE II

Sample Weight	Theoretical % N	Actual % N Found
0.2004 gm.	2.85	2.98
0.2000 gm.	2.84	2.86
0.2001 gm.	2.84	2.96
0.2003 gm.	2.85	2.89

Determination of the water of hydration.

In justification of the above statement the water of hydration was previously determined. It was found that oven heat at 80 degrees C. for one hour decomposes the glucoside, and sulphuric acid—vacuum desiccation removes slightly more than one molecule of water of crystallization progressively during a period of ten days. Obviously, the latter process, because of time duration and inconsistency, is not analytically applicable.

The method which proved to be most satisfactory was the Dean and Stark saturated water-xylol distillation process which gave the following data:

TABLE III

Sample Weight	Theoretical Volume based upon 2 H_2O	Actual Volume Collected
9.0035 gm.	0.6504 ml.	0.65 ml.
7.0012 gm.	0.514 ml.	0.52 ml.

In each case the sample was mixed with 25 ml. of water saturated xylol and the mixture heated in a CO_2 flask connected to a Dean and Stark tube and condenser. It is quite evident that the experimental results indicate that the commercial substance possessed two molecules of water of crystallization in contrast to three molecules as generally reported in the literature.

Hydrolytic constants.

Inasmuch as a variation has been previously noted in the rate of reactivity of amygdalin with the respective alkali solutions, investigations were instituted to determine the hydrolytic constant in each case. Approximately 8 gms. of amygdalin were dissolved in enough of the solution to total 100 mls. A portion of each solution was viewed in a 200 mm. water-jacketed polariscope tube in a saccharimeter with the results noted below.

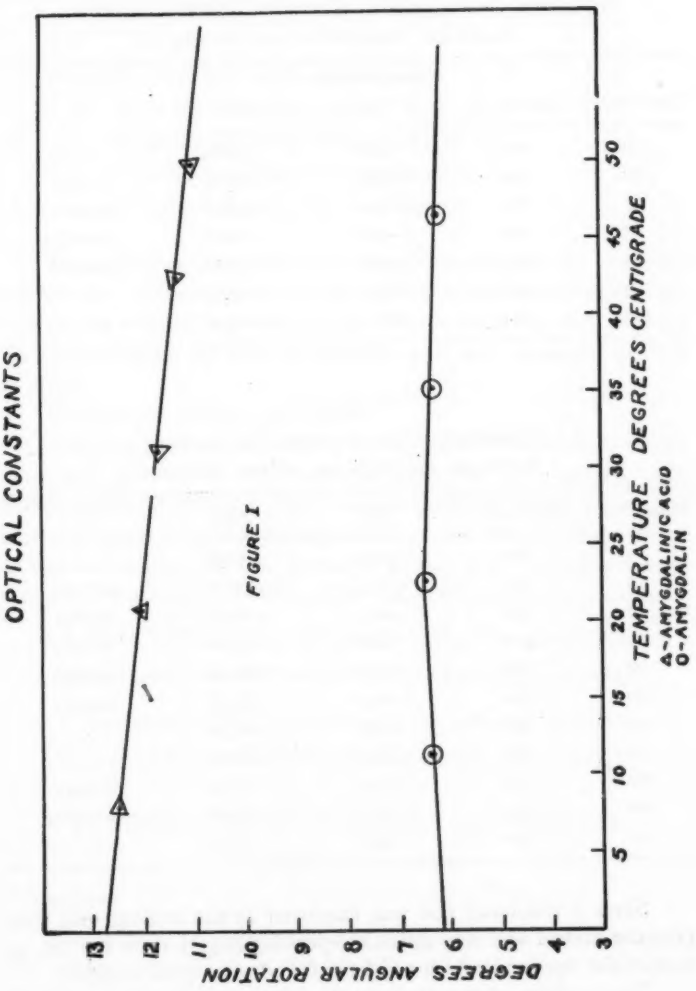

OPTICAL CONSTANTS
DEGREES ANGULAR ROTATION
13
12
11
10
9
8
7
6
5
4
3
5
10
15
20
25
30
35
40
45
50
TEMPERATURE DEGREES CENTIGRADE
Δ-AMYGDALINIC ACID
O-AMYGDALIN

FIGURE I

TABLE IV

HYDROLYTIC CONSTANTS WITH SATURATED $Ba(OH)_2$ SOLUTION

10.2480 gm. Amygdalin q. s. to 100 mls.

Time (min.)	Degrees C.	Direct Reading °SS	AR°	K
0	24.2	—30.0	—50.76	.....
30	50.0	—33.2	—56.18	0.01858
45	56.0	—35.0	—59.22	0.03611
65	50.0	—36.5	—61.76	0.008651
80	46.0	—37.0	—62.61	0.04661
95	47.0	—37.0	—62.61	.....
110	50.0	—37.5	—63.45	.....

TABLE V

HYDROLYTIC CONSTANTS WITH N/2 SOLUTION

8.0216 gm. Amygdalin per 100 mls. Solution

Time (min.)	Degrees C.	°SS	AR°	K
0	28.0	—24.0	—51.88	.....
5	55.0	—27.0	—58.37	0.1267
10	55.0	—28.1	—60.75	0.07831
15	55.0	—28.8	—62.26	0.07249
20	56.0	—29.0	—62.69	0.02656
30	56.0	—29.4	—63.56	0.03385
40	54.0	—29.4	—63.56	.....
70	55.0	—29.4	—63.56	.....
100	53.0	—29.9	—64.64	0.02311
160	56.0	—30.0	—64.85	0.003604
—	64.0	—30.4	—65.72	.....

Since a resistance coil was employed as the heating unit temperature control was very difficult depending largely upon the rate of flow of the tap water which was later found to be quite variable.

The reaction, obviously proceeds in accordance with the mathematical expression for a monomolecular reaction and is catalyzed by the presence of the hydroxyl ion.

$$K = \frac{2.303}{ty\text{-}tx} \log_{10} (Ax - A) - \log_{10} (Ay - A)$$

where A is the final angle of rotation in °A. R.;
Ax is the preceding reading in °A. R.;
Ay is the following reading in °A. R.;
tx is the preceding time in minutes;
ty is the following time in minutes.

It is apparent from the experimental results that the reactivity between the reactants in the KOH solution is much more rapid than in a similar solution with $Ba(OH)_2$. The products in each case are the corresponding salts of amygdalinic acid with ammonia evolved as a gas.

Products of alkaline hydrolysis.

The final products of alkaline hydrolysis which have been briefly mentioned previously are ammonia and the salt formed with amygdalinic acid from the alkali used in the hydrolyses. They arise as a consequence of the decomposition of the nitrile radicle in the amygdalin molecule to the carboxyl group, with the simultaneous evolution of ammonia. The reaction may be indicated as follows:

1. $C_6H_5CH.(CN).O.C_6H_{10}O_4.O.C_6H_{11}O_5 + 2HOH$
$= C_6H_5CH(COOH).O.C_6H_{10}O_4.O.C_6H_{11}O_5 + NH_3$

2. $C_6H_5CH(COOH).O.C_6H_{10}O_4.O.C_6H_{11}O_5 + MOH$
$= C_6H_5CH(COOM).O.C_6H_{10}O_4.O.C_6H_{11}O_5 + H_2O$

B. PRODUCTS OF ALKALINE HYDROLYSIS

1. Amygdalinic Acid

Preparation

The general procedure followed is indicated on the flow sheet. The process depends upon the solubility of the lead salt * in water and the subsequent decomposition by hydrogen sulphide.

*Proctor (11) in summarizing observations, states that "none of the amygdalates insofar as they had been examined, were insoluble but the amygdalate of lead." This statement was found to be erroneous.

FLOW SHEET OF THE AMYGDALINIC ACID PREPARATION

AMYGDALIN

$Ba(OH)_2$ solution

Barium + Ammonia

Amygdalinate

dil. H_2SO_4

Amygdalinic Acid + $BaSO_4$ ppt.

excess H_2SO_4

Basic Lead Carbonate

Lead $PbSO_4$ ppt.

Amygdalinate $2PbCO_3 : Pb(OH)_2$

+

H_2S

Amygdalinic PbS.

Acid

$$C_{20}H_{27}NO_{11} + 2H_2O = C_{20}H_{28}O_{13} + NH_3$$

13.11450 gm. of amygdalin were boiled cautiously with 150 mls. of the saturated $Ba(OH)_2$ solution in a 250 ml. Erlenmeyer flask over a wire asbestos gauze until the evolution of ammonia had ceased (approximately three-quarter hour). The solution was permitted to cool. A calculated quantity of 10 per cent. H_2SO_4 was added to totally precipitate both the combined and uncombined barium ion with a slight excess of the reagent remaining. The mixture was then digested at a low temperature upon the water bath for fifteen minutes to increase the size of the $BaSO_4$ crystals, filtered, washed with water, and to the filtrate enough basic lead carbonate ($2PbCO_3.Pb(OH)_2$) in excess was introduced to totally neutralize the acidity due to the remainder of the sulphuric acid as well as produce the soluble amygdalinate of lead. After digestion for half an hour upon the water bath, the solution was filtered with suction, washed well with water until negative in reaction, to congo red test paper and cooled to room temperature. Hydrogen sulphide was passed into the solution until it was saturated. The operation was continued at a slightly elevated temperature for fifteen minutes, until, upon settling of the lead sulphide, the supernatant liquid showed no further colloidal precipitate. The solution was filtered, the precipitate thoroughly washed with distilled water and the total filtrate

was transferred to an evaporating dish. The evaporation was conducted until a syrupy consistency of the liquid was obtained. It was then transferred to a smaller vessel with a few small portions of water, evaporated until viscous and placed in a vacuum desiccator over sulphuric acid to thoroughly dehydrate. This operation may require several days for completion after which the resulting white mass may be pulverized.

Yield, 12.368 gm. equivalent to 94 per cent.

A portion of the compound, when dissolved in water, should show a weakly acid reaction to congo red test paper, should be free from heavy metals and free from sulphate ion.

Physical Properties

The physical constants of the anhydrous acid, which were subsequently determined, were found to be:

M. P. = 125 degrees to 128 degrees C. with slight decomposition.

[α] d° from water is —71.92 degrees (Fig. I). This value is obtained from the graph by extrapolation, and from the observed values, read in a two-decimeter tube with a weight of 2.2177 gm. of sample q. s. to 25 ml.

TABLE VI

Temp. °C.	Reading °SS	°SS to °AR (°SS x 0.3468)
8.4	—36.0	—12.485
21.	—34.8	—12.068
31.4	—33.8	—11.722
42.6	—32.7	—11.340
50.0	—32.0	—11.098

$$\frac{°SS \times 0.3468 \times 100}{2 \times 8.8708} = \frac{-t}{d} = [a]$$

Contrary to the data submitted by Walker, the compound thus isolated evidently does possess decided optical activity. Perhaps it can be most easily explained on the basis that when the substance is totally freed from alkali and the asymmetric carbon atom again assumes the same configuration that it possessed in the amygdalin molecule, but since it is now attached to the carboxyl group instead of a nitrile group its optical activity is altered.

During the process of early dehydration, traces of crystallization can be observed. However, when viewed microscopically, the sub-

stance exhibits no crystalline form that can be conclusively defined. It absorbs moisture from the air with avidity and liquifies. It is soluble neither in cold nor boiling absolute alcohol, but slightly soluble in 95 per cent. alcohol and slightly hydrated acetone and totally insoluble in ether. When the aqueous solution is evaporated to dryness upon the water bath a porcelain-like residue remains which is very difficultly removed.

Chemical Properties

The composition $C_{20}H_{28}O_{13}$ $(OH)_7C_{12}H_{14}O_4C_7H_6CO_2H$. as determined by Liebig and Wöhler from the anhydrous barium salt by combustion with CuO is:

		Theoretical in 100 pts.	Found
40 atoms Carbon,	3057.480	45.519	45.335
52 atoms Hydrogen,	324.469	4.814	5.029
24 atoms Oxygen,	2400.000	35.466	36.458
1 atom Barium,	956.880	14.199	13.178
1 atom Bar. Amyg.	6738.829		

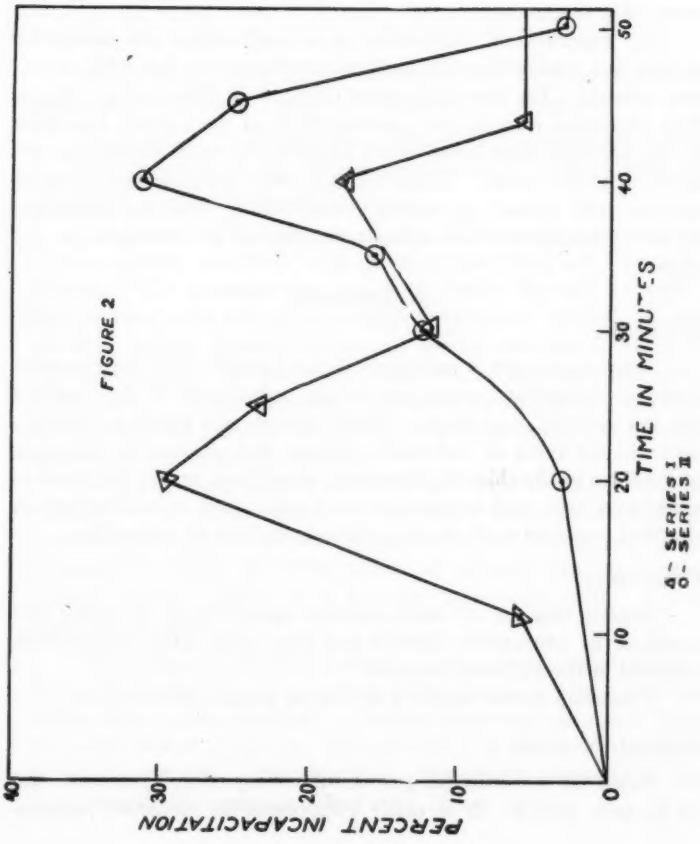
When boiled with a dilute hydrochloric acid solution it progressively yields mandelic acid and when heated with MnO_2 in the presence of a low concentration of sulphuric acid, it evolves, by distillation, carbonic and formic acids as well as benzaldehyde. The salts are mostly gummy, such as the bariumamygdalinate ($BaC_{20}H_{26}O_{13}$). Amygdalinic acid, according to Schiff, forms the following compounds: 1. Tetracetylamygdalinic acid, upon heating with acetic acid anyhydride at 70 to 80 degrees C. ($C_{28}H_{36}O_{17} + H_2O = C_{20}H_{24}(C_2H_3O)_4.O_{13} + H_2O$, representing a leaflike glassy mass, and somewhat soluble in water, but much more readily soluble in alcohol and ether. 2. Heptacetylamygdalinic acid, upon boiling of amygdalinic acid with acetic acid anhydride. $C_{34}H_{42}O_{20} = C_{20}H_{21}(C_2H_3O)_7\ O_{13}$;,a starchlike powder, insoluble in water, fairly soluble in ether, and very readily soluble in alcohol.

Physiological Properties

In order to establish the toxicity of amygdalinic acid, experiments were conducted with the daphnia as the test animal, as suggested by the senior author.

Since this organism is transparent and possesses the essential functions of higher animals, its adaptabilities to physiological testing are innumerable. Furthermore, the effect of a particular substance upon several organs can be obtained and noted simultaneously. Previous work upon the standardization of numerous drugs of which strychnine and aloes may be cited to substantiate this statement.

One ml. of a 0.3 per cent. aqueous solution of amygdalinic acid was introduced into each of nine tubes containing a pair of animals; the tenth tube was reserved as a control.



After a lapse of approximately twenty minutes with few exceptions, practically all the animals exhibited spasmodic movements. Upon observation and comparison with the control, the breathing organs and the heart were noticeably affected in that the functional activity of each system had been considerably impaired. The animals continued to live, however, for twenty-four hours longer.

Upon repetition with greater concentration (2 per cent.) the incapacitation of all the organisms occurred within one hour, and as is obvious from the graph (curve 1), the maximum number of fatalities were observed within twenty-five minutes. These animals were three weeks old.

An experimental duplication as a confirmation of the above results was conducted with the same concentration but with thirty-two animals. The resultant curve (No. 2) is illustrated in Fig. 2. The organisms were of the parental stock of the animals employed in the previous experiment which explains, in some degree, the delayed paralyses point. These animals were approximately sixteen days old and seemed to possess greater vitality than the organisms formerly employed. The animals weighed as an average 2 to 2.5 mgms.

2. Ammonia

Occurrence

Ammonia occurs in nature mainly as a result of the fermentative decay of nitrogenous plant and animal substances; it thus reaches the soil and the atmosphere. Small amounts are found in the rain water in the form of carbonates, nitrites and nitrates; in the urine in the form of the chloride, phosphate or sulphate, and in the blood—either as a free base or dissolved as a salt. It is often liberated in chemical reactions such as the alkaline hydrolysis of amygdalin.

Preparation

Readily obtained in small amounts upon heating of strong ammonia or of ammonium chloride and lime, pure NH_3 is said to be obtained in the following manner:

Potassium nitrite heated with caustic potash, iron and zinc

Physical Properties

Ammonia is a colorless gas of suffocating odor, lighter than air (0.59 spec. grav.). It is easily compressed by increased pressure

or lowered temperature to a colorless liquid. It is readily dissolved by liquids, particularly water, which absorbs 1148 times its own volume at 0 degrees C. and 760 mm. pressure.

Chemical Properties

Ammonia forms a multitude of salts, inorganic and organic, of diagnostic character. Small amounts of ammonia are detected with Nestler's reagent (an alkaline solution of mercuric iodide—potassium iodide), producing a yellow or red color, depending upon the amounts of ammonia present.

Physiological Properties

Permeability: As a liquid, dissolving the lipoid substance, ammonia penetrates very easily into the plant and animal cells.

Local effect: Ammonia causes irritation of mucous membranes, increased secretion of the salivary gland as well as the general local symptoms of caustic alkalies (18a).

Resorption: When introduced into the stomach, ammonia may be very quickly resorbed. Cramps have been observed after a few minutes. The ammonia ions penetrate readily through the wall of the intestinal canal and, as we shall describe below, through the walls of other tissues, causing widespread lesions and hemolysis of red blood corpuscles. In mammals, toxic effects are the simultaneous occurrence of strong symptoms of irritation and central paralysis. The secretion of saliva is increased, vomiting occurs almost regularly, even after subcutaneous injection.

Head and neck muscles show the first light twitching, followed suddenly by a violent tetanus of the entire voluntary musculature, continued to death, or followed by periodic clonic convulsions, tremor of muscles—return of reflex action to stimuli, causing renewed cramps, finally followed by a comatose state—and death. Further data, see on table.

Concerning its effect on daphnia: Ammonia in concentrations of 0.1 cc. 28 per cent. to 40 cc. culture water caused asphyxia and death within fifty to sixty seconds, in higher concentrations much faster; in concentrations of 0.1 cc. (28 per cent.) to 10 cc. culture water practically instantly. The symptoms corresponded generally to those characteristic for ammonia effect upon higher animals.

EFFECT OF AMMONIA AND AMMONIUM SALTS

Substance	Animal	Wgt.	Form of Application	Toxic Dose	L. D.	Symptoms	Literature	Remarks
NH_3	Cat		Inhalation	2.5 vol. %	1-2 vol. %		Heffter	
NH_3	Cat	3 kg.	Oral		0.75 gm.		Heffter	
$(NH_4)_2CO_3$	Rabbit		Oral	20 cc. 5% sol.		Vomiting	Sollmann	
$(NH_4)_2CO_3$	Dog		Intravenous	0.1 gm.			Sollmann	
$(NH_4)_2CO_3$	Frog		Subcutan.	0.4 gm.			Sollmann	
NH_4Cl	Guinea pig	p. kg.	Subcutan.		0.30-0.355 g. per kg.		Sollmann	
	Guinea pig	p. kg.	Intravenous		2.150 g.		Sollmann	
	Mouse	p. kg.	Subcutan.		0.59		Sollmann	
	Rabbit	p. kg.	Intravenous		0.159		Sollmann	Death in appr. 1½ hrs.
	Rabbit		Intravenous		0.319		Sollmann	Death in appr. 2½ hrs.
	Frog		Into lymph sac	0.15 gm.	0.19		Sollmann	
	Frog		Intravenous				Sollmann	

EFFECT OF AMMONIA

Substance	Animal	Weight		Lethal Dose	Symptoms	Authors	Remarks
NH_3	Rat	250 gr.	Orally, tube (5 cc. with syringe)	Appr. .25 pro kgr.	Immediately: No striking symptoms. Soon after: Saliva flow. Animal drops head to paper base and becoming weaker. Breathing becoming labored. After 14 minutes: Tetanic-like cramps in body. After 17 minutes: Tetanic-like cramps repeated. After 18½ minutes: Violent spasms. Animal lying on side. Heartbeats 150 per minute. After 22 minutes: Rapidly repeating spasms and clonic cramps. Insensitive to mechanical irritation. Breathing very much slower. Animal losing body control. After 30 minutes: No respiration, no heartbeat—cyanosis. After 31 minutes: Dead. Post Mortem: Stomach-wall—alkaline, lesions. Tissue exposed: Intestine—Duodenum lesions. Small intest. lesions. Pancreas—badly bloodshot, lesions. Smells of Ammonia: Liver—lesions. Kidney—lesions. Spleen (almost blue)—lesions. Bladder slightly alkaline—lesions.	Viehoever and Mack	Administered 4 hours after feeding.

VI

PRODUCT OF CONCENTRATED ACID HYDROLYSIS

L-Mandelic Acid

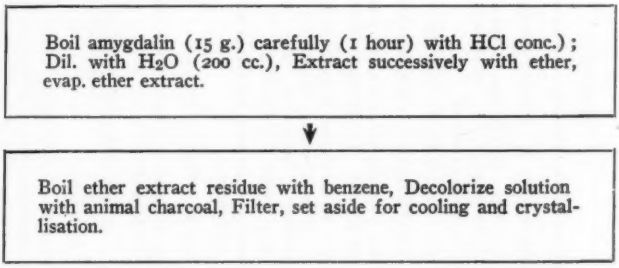
Occurrence

This substance does not occur free in nature so far as we know.

Preparation

Wöhler (Ann. d. Chem. 66, 240) prepared l-mandelic acid by heating amygdalin with fuming hydrochloric acid.

The following process, indicated in the flow sheet, gave a satisfactory product.


Boil amygdalin (15 g.) carefully (1 hour) with HCl conc.); Dil. with H_2O (200 cc.), Extract successively with ether, evap. ether extract.
Boil ether extract residue with benzene, Decolorize solution with animal charcoal, Filter, set aside for cooling and crystallisation.

Physical Properties

L-mandelic acid forms colorless, shining scalelike crystals. Melting at 132.8 degrees C.; soluble to 8.6 per cent. in water (20 degrees C.) also readily soluble in ether and alcohol. It is l-rotatory, the specific rotation being 146.6 degrees at 14 degrees C.

Chemical Properties

The cinchonine salt permits differentiation of l-mandelic acid from d-mandelic acid. Oxidation changes mandelic acid into benzoic acid, reduction (heating with HI) into phenylacetic acid.

Physiological Properties

No information has been found in literature for the physiological action of l- and d-mandelic acids. The inactive or racemic form, action of l- and d-mandelic acids. The inactive or racemic form (19a)), acted as a diuretic; when given to a dog, it passed largely unchanged into the urine.

EFFECT OF L—MANDELIC ACID UPON RATS

No.	Animal	Sex	Weight	Form of Application	Amount	Symptoms
1	Rat	Female	170 gm.	Subcutaneous Peritoneal	2 cc. of 6.4% Aq. Sol. = 0.128 gm.	Within 10 minutes: Eyes partially closed. Restlessness of body.
2	Rat	Female	190		3 cc. of 6.4% Aq. Sol. = 0.199 gm.	After 20 minutes: Waviness of diaphragm. Spasmodic contraction in abdominal portion. Respiration rate slowed, 72 beats per minute. Twitching of body. After 30 minutes: (1) drinks water. Within 24 hours: Both recovered.

EFFECT OF L—MANDELIC ACID UPON DAPHNIA

No.	Concentr.		Age	Time	General	Locomotory S.	Digestive S.	Respiratory S.	Circulatory S.	Remarks
	%	Amt.								
1	0.6	0.5cc		¾ min.	Swimming on back	Little intermittent cramps like action of antennae	Paralysed			
				4 min.	Swimming on back, on on bottom			Stopped	Without function	Dead
2	0.48	0.5cc		5 min.	Swimming on bottom on back		Paralysed	Stopped		No Laxative action
				6 min.	Swimming on bottom on back					Dead (?)
3	0.36	0.5cc		1 min.	Swimming on bottom on back					
				2 min.				Paralysed		
				6 min.			Paralysed		Heart dilated 132 bts. p. min.	Optical S. paralysed
				10 min.					46–60 bts. p. min	Optical S. paralysed
				13 min.					0 bts. p. min.	Dead (?)

EFFECT OF L—MANDELIC ACID UPON DAPHNIA

(FRESH SOLUTION: 0.242%)

Time Minutes	1	2	3	4	5	6	7	8	9	10
After ½			Swimming up side down							
After ¾							Swimming up side down	Swimming up side down		
After 1	Swimming up side down				Swimming up side down	Swimming up side down			Swimming up side down	Swimming up side down
After 2		Swimming up side down								
After 3	On bottom				On bottom	On bottom				
After 4	Motionless	Motionless		Motionless						
After 5							Motionless	Motionless		
After 6			Motionless							
After 7							Motionless			In motion Intermittently
After 7½					Motionless	Motionless				

EFFECTS OF L—MANDELIC ACID UPON DAPHNIA—(Continued)

Time Minutes	1	2	3	4	5	6	7	8	9	10
After 8									In motion Intermittently	
After 11½					Motionless		Motionless	Swimming Intermittently		
After 13						Swimming Intermittently				
After 14				Motionless						
After 18	† (?)	† (?)							In motion Intermittently	In motion Intermittently
After 21½								† (?)	Antennae in tremor	
After 25					motionless	In motion Intermittently	†			
Within 28	†	†	†	†					†	†
Within 30	†	†	†	†	†	†	†	†	†	†

† = dead.

EFFECT OF L—MANDELIC ACID UPON DAPHNIA

(ONE DAY OLD SOLUTION: 0.2421%)

No.	Concentra. %	Concentra. Amt.	Age	Time	General	Locomotory S.	Digestive S.	Respiratory S.	Circulatory S.	Remarks
1	0.242	0.5	17 days							No immediate effect
	1 day old sol.			17 min.	Swimming sideways			Slowed down		
				21 min.	Coming to bottom frequently		Digestive canal almost emptied			
				37 min.						Motionless (Dead?)
2	0.242 1 day old sol.	0.5	17 days	17 min.		Greatly slowed down	Digestive canal almost emptied	Slowed down		
				47 max.						Motionless (Dead?)

Aqueous solutions of l-mandelic acid in concentrations as low as ± 0.27 per cent. shows definite physiological effects, within one minute; the daphnia turning sideways, downward, swimming backward, with head down and finally upside down. Within few minutes paralysis of digestion, respiratory and circulatory system may be observed, death occurring usually within less than an hour, unless the animals are transferred into normal culture water. In solutions of 0.12 per cent., the animals remained active for at least three hours, though showing the temporary effect of paralysis of respiration and other symptoms. Concentrations of 0.36 to 0.67 per cent. showed the like symptoms more rapidly—and death within a few to thirteen minutes, according to concentration. A 0.242 per cent. solution, after standing twenty-four hours, showed no striking immediate physiological effects. These effects became evident, however, after seventeen minutes, leading to death within one-half to three-quarters of an hour.

VII

COMPARATIVE PHYSICAL PROPERTIES

Physical Constant	Amygdalin	Amygdalinic Acid	L—Mandelic Acid	Benzaldehyde
1. Crystalline Form	Rhombic (f. alc.)	Indefinite	Laminated plates from benzene	—
2. Melting Point	214°—216° C.	125°—128°C.	132°C	—13.5°C.
3. Optical Rotation	—38.47° at 0°C.	—71.92° at 0°C.	—146.6° at 19°C.	—
4. Solubilities	in grms. per 100 cc. of solvent.			
(a) Water	8.3 at 10°C.	1.1 or more	8.64 at 20°C.	0.33
(b) Alcohol (Absol.)	0.11 at 10°C.	0.0	S.	∞
(c) Ether (Anhyd.)	0.0	0.0	S.	∞

COMPARATIVE CHEMICAL COMPOSITION

Amygdalin	Amygdalinic Acid	L—Mandelic Acid	Benzaldehyde
$C_{12}H_{21}O_{10}—O—CH\begin{cases}C_6H_5\\CN\end{cases}$	$C_{12}H_{21}O_{10}—O—CH\begin{cases}C_6H_5\\COOH\end{cases}$	$HO—CH\begin{cases}C_6H_5\\COOH\end{cases}$	$O=C\begin{cases}C_6H_5\\H\end{cases}$

VIII

SUMMARY

Amygdalin

Hydrolysis Products	Hydrolysis Products
With ENZYMES (EMULSIN) or With DILUTE ACIDS	With ALKALI: KOH or $Ba(OH)_2$
$C_{20}H_{27}NO_{11}+^{2}H_2O=HCN+C_7H_6O+2C_6H_{12}O_6$	$C_{20}H_{27}NO_{11}+2H_2O=C_{20}H_{29}O_{13}+NH_3$
Amygdalin — Hydrocyanic Acid — Benzaldehyde — Glucose	Amygdalin — Amygdalinic Acid — Ammonia

Hydrolysis Products
With Conc. Acids (Fuming Hydrochloric Acid)

$C_{20}H_{27}NO_{11}+4H_2O=C_8H_8O_3+2C_6H_{12}O_6+NH_3$
Amygdalin — L. Mandelic Acid — Glucose — Ammonia

CONCLUSIONS

The biochemical research of amygdalin, involving the physical, chemical and physiological study of amygdalin as well as its products of hydrolysis, has yielded interesting new physical, chemical and physiological results.

1. Commercial amygdalin was found to contain two (2) molecules of water of crystallization—in contrast to three molecules, as reported in the literature.

2. The hydrolytic conversion of amygdalin has been confirmed. Enzyme (emulsin) and dilute acid convert it to hydrocyanic acid; benzaldehyde and glucose. Alkalies cause its decomposition into amygdalinic acid and ammonia. Concentrated acid (hydrochloric), forming amygdalinic acid and ammonia as intermediary products, hydrolyzes amygdalin finally to l-mandelic acid, ammonia and glucose.

3. The purity and quantity of the amygdalinic acid produced during the alkaline hydrolysis may be indirectly measured by the amount of ammonia liberated.

4. Amygdalinic acid may be isolated by decomposing its barium salt with an excess of sulphuric acid, neutralizing with basic lead carbonate, and then decomposing the soluble lead amygdalinate to free the amygdalinic acid.

5. Amygdalin exerts its action obviously only to the extent, in amount and speed, as it is hydrolyzed to hydrocyanic acid and benzaldehyde. This effect, previously observed in higher animals (vertebrates) and particularly in plant eaters, has now been demonstrated also for the crustacean daphnia. In 2 per cent. and 4 per cent. solutions of amygdalin in culture water death was only observed, as a rule, after sixteen to twenty-four hours—due, no doubt, to the slow hydrolytic action by digestive enzymes.

6. The laxative, cramp, narcotic and fatal action of hydrocyanic acid on daphnia was readily observed in a 2 per cent. culture water solution of amygdalin, hydrolyzed by the addition of emulsin. If 0.1 per cent. emulsin was added, death occurred in all (fourteen) animals in sixty to seventy minutes; fourteen normal daphnia, then, placed into the very same solution, died within thirty to fifty seconds, due, no doubt, to the presence of hydrocyanic acid accumulated. If 1 per cent. emulsin was added to the 2 per cent. amygdalin solution in culture water, the animals (fourteen) died within five to seven

minutes, showing the marked symptoms characteristic of poisoning of hydrocyanic acid.

7. Benzaldehyde appears only comparatively harmless, as reported in some references of the literature, when quickly oxidized in the body to benzoic acid. Otherwise, obviously through partial solution of cell lipoids, it will cause toxic effects of narcosis, spasmic and depressed breathing and death of rats, upon oral administration of 3.5 cc. per kg. and subcutaneous (peritoneal) injection of 3 cc. per kg. of body weight. In a 0.01 per cent. solution in culture water, after like symptoms, daphnia died within one to two hours, although the animals invariably recovered from initial paralysis or cramps—when the solution was exposed to air and oxidation in a very thin layer. In concentrations of 0.4 to approximately 0.8 per cent. benzaldehyde-culture-water solutions, the animals were completely paralyzed within five to twenty seconds, depending upon the concentration.

8. Amygdalinic acid, showing no marked toxicity to daphnia submerged in a 0.3 per cent. solution for several hours, caused paralysis and death in 2 per cent. concentration within thirty to fifty minutes.

9. *Ammonia,* in amounts of the lethal oral dose reported for cats (0.25 gm. per kg.) also proved fatal to a rat, with the progressive symptoms of (1) increased secretion of saliva, (2) tetanic spasms or convulsions, followed by (3) coma and (4) death—within one-half hour. Added in concentration of 0.1 cc. (28 per cent.) to 10 cc. culture water, ammonia caused instantaneous death, in one-quarter that concentration, cramps, asphyxia and death within fifty to sixty seconds.

10. *L-Mandelic Acid,* while causing only temporary narcosis, depression of the respiration and twitching of the body of rats, upon subcutaneous (peritoneal) administration of 1 gm. per kg., showed definite physiological effects in somewhat higher concentrations upon daphnia, placed in such solutions. Partial paralysis of the digestive, respiratory and circulatory systems may be observed within a few minutes, death within less than an hour in freshly made solutions of 0.24 to 0.27 per cent. solutions. In a day old solution the characteristic symptom and death are delayed; in higher concentrations (0.3 to 0.67 per cent.) death occurred within a few to thirteen minutes, according to the concentration of the fresh solutions.

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SCIENTIFIC AND TECHNICAL ABSTRACTS

Compiled by Linwood F. Tice, M. Sc.

Dinitrophenol Not Acceptable for N. N. R. Am. Med. Assoc., Council on Pharmacy & Chemistry *J. Am. Med. Assoc. 105,* 31 (1935). Dinitrophenol (2, 4) has been extensively studied by numerous workers with somewhat varied results. It is estimated that about 100,000 persons in this country alone have since its introduction used this drug for the treatment of obesity. About twenty commercial concerns are furnishing dinitrophenol or mixtures in which this drug is the active principle. To date six persons are known to have been fatally poisoned by the use of this drug and in some of these cases the dose was not in excess of the usual dose recommended, namely, 5 mg. per kilogram. Toxic symptoms include dizziness, dyspnea, fatigue, pyrexia and excessive perspiration. Death may result within twenty-four hours.

If the required dosage to accomplish satisfactory loss in weight is used as an index of the proper dosage, many further fatal poisonings are sure to occur.

In view of the close supervision necessary for the proper administration of this drug and the danger of widespread improper use, the Council voted against its inclusion in the N. N. R. This action is in harmony with that taken in Canada where, for example, it may not be sold for the treatment of obesity. England and Germany have taken similar regulatory steps to properly limit its sale.

Preservation of Anesthetic Chloroform. A. Lissierici-Draganesco *J. Pharm. Chim. 21,* 533-538 (1935) through *Brit. Chem. Abstr. B 54,* No. 34, 748 (1935). Pure chloroform for anesthesia containing 1 per cent. ethyl alcohol was stored in well sealed, colored tubes of small capacity in the dark at 22-26 degrees C. for four years. No chlorine, hydrochloric acid, carbonyl chloride, aldehydes, or organic impurities were present upon final examination. Samples exposed to daylight and, in particular, direct sunlight were found to rapidly generate hydrochloric acid.

Permanent Aqueous Microscopic Mounts. H. R. Smith, *J. Ind. & Eng. Chem. Anal. Ed., 7,* 286 (1935). The need for a satisfactory

sealing material for permanent aqueous mounts is discussed and a number of substances not proving satisfactory are cited.

The author found that a mixture of anhydrous lanolin in which 20 per cent. rosin was incorporated by heat provided an excellent sealing material. It is suggested that the water used in preparing such a mount contain about 0.2 per cent. by volume of 40 per cent. formaldehyde solution. The sealing is accomplished by applying the melted material to the edge of the cover glass whence it quickly congeals and provides an excellent seal.

Differentiation of Chloroform and Carbon Tetrachloride. N. Schoorl, *Pharm. Weekblad* (June 29, 1935) through *Squibb Abstract Bulletin 8,* No. 38 (1935). Fehling's solution is reduced upon boiling it with chloroform but not by carbon tetrachloride. On warming one drop of the liquid to be tested in a stoppered tube carbon tetrachloride will be oxidized by the air and give off some chlorine but chloroform will not. If the tubes are then cooled, a few cc. of water shaken in them, and starch potassium iodide solution added the carbon tetrachloride sample will turn blue.

Estimation of Chloramine in Water Supplies. P. D. McNamee. *Ind. Eng. Chem. Anal. Ed. 7,* 333 (1935). The widespread use of chloramine (NH_2Cl) instead of chlorine in the disinfection of water supplies renders a distinctive test for chloramine desirable. The o-tolidine test for residual chlorine is not altogether satisfactory due to the interference by the nitrite ion which may be expected in chloramine treated water.

Above pH 8.5 chloramine exists entirely as monochloramine and below pH 4.4 only nitrogen trichloride is produced according to the equation $3NH_2Cl + 2H^+ \rightarrow 2NH_4^+ + NCl_3$, two-thirds of the nitrogen of the chloramine being converted into ammonium ion.

The determination depends upon ascertaining the amount of ammonium ion liberated upon acidification, the ammonium ion liberated being determined colorimetrically using Nessler's Reagent, and a blank for ammonia nitrogen being run on an alkaline sample. The chloramine-chlorine content is then obtained by multiplying the observed increase in ammonia-nitrogen by $3/2 \times 35.5/14$ or 3.8.

The presence of large amounts of free ammonia reduces of course the precision of the test and consequently it is not of value

when applied to sewage or sewage plant effluent. It is, however, quite advantageous in distinguishing free chlorine and chloramine-chlorine in a sample of water.

Employment of Potassium Ferrocyanide in Standardization of Dilute Potassium Permanganate. E. J. deBeer and A. M. Hjort *Ind. Eng. Chem. Anal. Ed. 7,* 120 (1935). The advantages offered by potassium ferrocyanide as a primary standard in permanganimetry, particularly in microchemical analysis, as compared with sodium oxalate are, first, its solutions need not be freshly prepared; second, it has a high equivalent weight (an error of 37 mg. in weighing one equivalent of the anhydrous salt would cause an error of only 0.01 per cent.); and lastly, heat is not required in the titration. The end-point of the titration is determined by the use of erioglaucine as an indicator as suggested by Knop (*Z. Anal. Chem. 77,* 111 1929), the color change being from apple green to amber. The titration is carried out in the presence of sulfuric acid (0.1-1.0 N.) with continuous agitation and an indicator correction must be applied to the potassium ferrocyanide titer. Dilute solutions of potassium ferrocyanide suitable for standardization use were found to change only 0.03 per cent. in normality in twenty-two days.

A Procedure for Classification of Hydrocarbons. S. P. Mulliken and R. L. Wakeman. *J. Ind. Eng. Anal. Ed. 7,* 275 (1935). The method presented in this article was devised to replace that used for Genus IX (hydrocarbons) by Mulliken ("Identification of Pure Organic Compounds" Vol. I) in view of the growing scientific and commercial importance of this class of compounds.

The system now used divides the liquid hydrocarbons into three groups, depending upon their density and their reactivity with bromine, nitric acid and sulfuric acid. By means of density the aromatics and other heavy hydrocarbons are classified apart from the acyclics and lighter cyclics which are divided into two groups upon the basis of their behavior with the reagents named.

The new method divides the genus into a larger number of sections making it possible to classify a hydrocarbon of unknown structure according to the following scheme:

A—Gaseous hydrocarbons.
B—Liquid hydrocarbons.
1. Aromatics, etc.
2. Unsaturated nonaromatics other than alkenes.
3. Alkenes.
4. Cycloalkanes.
5. Alkanes.
C—Solid hydrocarbons.
1. Aromatics.
2. Nonaromatics.

The scheme developed utilizes miscibility with various selective solvents, bromide-bromate titration, density and boiling point (melting point for solids). A chart for experimental classification is given which should prove of considerable value in the qualitative determination of this class of compounds.

A Study of the Extraction of Ipecac Root. K. Steiger. *Pharm. Acta Helv. 10,* 59 (1935). Ground Cartagena ipecac was separated into various sized powders by a series of sieves and the alkaloid and ash content of each determined. The finer powders contained much more alkaloid and ash than the coarser.

Using a second series of ground drug of various degrees of fineness in which each powder represented the whole drug a series of experiments were conducted which gave the following results:

1. For concentrated alcoholic menstrua a very fine powder ought to be employed, while for lower concentrations of alcohol a No. 40 powder was satisfactory.

2. Concentrated alcohol, due to the slow penetration into the cell contents, required a much longer time than diluted alcohol in order to bring in solution all the alkaloids that under the given conditions could be extracted from the drug.

3. Active trituration of the drug with concentrated alcohol (that is, using intermittent pressure and agitation) and the renewal of the solvent three times raised the speed of extraction considerably while simple stirring produced no increase in extraction.

Experiments with Hydroxybenzoic-acid Esters for the Sterilization of Eye Drops. J. Thomann. *Pharm. Acta Helv. 10,* 103 (1935) No. 6/7. In recent years parahydroxybenzoic acid esters as, for

example, the methyl ester (Nipagin) and the propyl ester (Nipasol) and others have been recommended for their bacteriostatic effect for the preservation of pharmaceutical preparations, particularly for eye drops and solutions intended for injection.

The author reports the results of a series of studies wherein eye drop formulas containing these esters in various amounts were purposely inoculated with various organisms and the bactericidal action of such esters determined over varying time limits. The results show that 0.05 per cent. of Nipasol required about three days to kill Staphylococcus Aureau and Bacillus Coli, but killed Aspergillus fungi in about twenty-four hours. A 0.2 per cent. solution of Nipagin killed the Staphylococcus, Bacillus Coli, and Aspergillus in twenty-four hours or less. Mesentericus and Aspergillus spores were not killed by 0.2 per cent. of Nipagin. Nipa-Sterilisator, a combination of parahydroxybenzoic acid esters, was found to be ineffective in concentrations of 0.1-0.15 per cent. at ordinary temperatures against all spores used. However, Mesentericus and cultured earth spores were killed if in addition to the presence of Nipa-Sterilisator in a concentration of 0.1-0.15 per cent. they were heated in free steam for thirty minutes. Even concentrations of Nipa-Sterilisator as high as 0.3 per cent., which is the limit of aqueous solubility, will not kill large numbers of native earth spores at ordinary temperatures, although some bacteriostatic effect is produced.

Inasmuch as in pharmaceutical practice no contamination is likely with earth spores in the number used, experiments were conducted which showed that if the earth spores were present in reasonable amounts complete sterilization was accomplished even with this very resistant type using 0.2 per cent. Nipa-Sterilisator and thirty minutes contact with free steam.

It seems that 0.15-0.2 per cent. of Nipa-Sterilisator causes no deleterious effects when injected or used in eye drops and the preparation appears to possess more intensive bactericidal action than the other similar esters heretofore used.

STATEMENT OF THE OWNERSHIP, MANAGEMENT, CIRCULATION, ETC., REQUIRED BY THE ACTS OF CONGRESS OF AUGUST 24, 1912, AND MARCH 3, 1933,

Of the AMERICAN JOURNAL OF PHARMACY, published monthly at Philadelphia, Penna., for October, 1935.

STATE OF PENNSYLVANIA, } *ss.*
COUNTY OF PHILADELPHIA, }

Before me, a Notary Public in and for the State and County aforesaid, Ivor Griffith, who, having been duly sworn according to law, deposes and says that he is the Editor of the AMERICAN JOURNAL OF PHARMACY, and that the following is, to the best of his knowledge and belief, a true statement of the ownership, management, etc., of the aforesaid publication for the date shown in the above caption, required by the Act of August 24, 1912, as amended by the Act of March 3, 1933, embodied in section 537, Postal Laws and Regulations, printed on the reverse of this form, to wit:

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Name of — Post-Office Address

Publisher: Philadelphia College of Pharmacy and Science, Forty-third and Woodland Avenue, Philadelphia, Pa.
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(Signed) IVOR GRIFFITH, *Editor.*

Sworn to and subscribed before me this twenty-third day of September, 1935.

(Signed) JOHN E. KRAMER, *Notary Public.*

My commission expires January 1, 1937.